Committee:
Dr. Jacobson, Chm.
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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-second Street
New York 17, N. Y.

#265

Application for Research Grant

Date: February 6, 1960

1. Name of Investigator: Gustave A. Laurenzi, M.D.

2. Title: Assistant Professor of Medicine
Coordinator of the Division of Respiratory Diseases

3. Institution & Address: Seton Hall College of Medicine
Medical Center
Jersey City 4, New Jersey

4. Project or Subject: Studies in bronchitis: A correlated investigation of the capacity of the lower respiratory tract to maintain sterility and the factors which interfere with the protective mechanisms involved. I. Human: A study of the bacterial flora of the upper and lower respiratory tract in normals (smokers and non-smokers) and chronic bronchitics (smokers and non-smokers). II. Animals: A study of the mechanisms whereby the respiratory tract clears bacteria: (a) demonstration of clearing; (b) demonstration (anatomical) of the sites of clearing and identification of the protective mechanisms involved, and (c) the study and comparison of the effects of cigarette smoke and sulfur dioxide on the clearing capacity.

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5. Detailed Plan of Procedure:
Headings refer to those in Section 4.
I. Human study: The examination of expectorated sputum for bacteria is misleading in that secretions from the lower respiratory tract are contaminated by oropharyngeal secretions. Studies have shown that in the normal, secretions in the bronchial tree below the carina are sterile. I am concerned with demonstrating this sterility by comparing the bacteriology of aspirates from the lower bronchial tree with the bacteriology of expectorated sputum and oropharyngeal secretions. I propose to study normal smokers and nonsmokers and patients with chronic bronchitis. Bronchial swabs and washings will be obtained under sterile conditions at bronchoscopy or by intubation with catheters (as in bronchography). All specimens are to be homogenized and plated out on appropriate media. (Please see section 10 for work done.)
II. Animal study: (a) In substance the method employed provides a means of administering quantitative doses of bacteria by aerosol to small animals for varying lengths of time and then observing what happens quantitatively to those bacteria that reach the lungs. Animals (rats, mice) are to be exposed to bacteria (pneumococci, staphylococci) in a large plexiglass cylinder. Broth cultures of the bacteria are atomized through a collision nebulizer. The cylinder acts as a mixing assembly and inhalation chamber. Groups of exposed animals are sacrificed at different time intervals after exposure; the lungs are removed aseptically and homogenized on a high speed tissue homogenizer; and bacterial counts are done on the homogenate by counting

colonies on agar pour plates. Time-concentration curves are constructed so that it is possible to predict within a range the number of bacteria present in an animal's lung at a certain time interval following the administration of a specific challenging dose. I am most interested in how fast the lung clears the bacteria, i.e. 1,000 organisms immediately after exposure; 0 organisms 24 hours later. (This year I have had the opportunity to work with this method following the intratracheal route of administration. By this route rats clear 40% to 50% of bacteria in 24 hours. Other work shows complete clearing of bacteria from the lungs of mice following aerosol administration.)

(b) The fluorescent-antibody technique is especially adaptable to identifying the major anatomical sites of clearing. In this method specific antibodies are made to a specific organism, and these antibodies are then conjugated with fluorescein isothiocyanate. A high titer fluorescein-antibody solution is employed as an immunochemical stain to sections of tissue. Examination of the tissue under the fluorescence microscope reveals the exact sites of penetration and phagocytosis. The identification of the protective mechanisms can be done by blocking the many known mechanical and immune factors. Ciliary action may be interfered with by anesthetics or alcohol; phagocytosis may be lessened by nitrogen mustards; humoral factors may be blocked by chemical means, i.e. blocking of complement by zymosan. The time-concentration curves will be worked out for animals under these various conditions, and comparisons will be made with the untreated animals.

(c) Different groups of animals will be exposed to cigarette smoke and sulfur dioxide. This will be done by administering bacteria in an aerosol with either smoke or sulfur dioxide and by giving bacteria to animals after more chronic exposure to these materials. Again, time-concentration curves will be determined as outlined in (a) and comparisons will be made.

6. Budget Plan:

Salaries	REDACTED
Expendable Supplies	1,944.00
Permanent Equipment	7,489.00
Overhead (15%)	2,525.00
Other	-
Total	REDACTED

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7. Anticipated Duration of Work: This grant request is for one year's support. The permanent equipment will make up an established aerosol laboratory. With the great versatility of the aerosol method I anticipate a much longer study of the many ramifications of this project.

8. Facilities and Staff Available: There will be adequate office and laboratory space at the medical school. There is ample space for the housing and care of laboratory animals, and culture media and basic bacteriological needs will be supplied by the hospital microbiology department. This project will require the part time participation of a pathologist and a thoracic surgeon. A bacteriology technician and a secretary will also be included.

9. Additional Requirements: The use of aerosolized cultures of bacteria requires maximum protection for personnel. Concerning this matter I have visited and consulted the Bacterial Warfare Center at Fort Detrick, Maryland, and the School of Public Health and Department of Industrial Hygiene at Harvard. The plan of this experiment and the protective measures we will use meet with their approval.

10. Additional Information (Including relation of work to other projects and other sources of supply): This year I have studied 25 patients by the method described in section 5, human study. All of these patients were bronchoscoped because of the suspicion of bronchogenic carcinoma. Four of these patients had chronic bronchitis, and all of them were moderate to heavy cigarette smokers for 25 years. Bronchial aspirates from the 21 non-bronchitics and one bronchitic were sterile. This indicates that any effect that smoking may have on the bronchial tree does not interfere with the normal clearing of bacteria. (This material is being prepared for a preliminary report in the New England Journal of Medicine.) A study of the mechanisms whereby the lower respiratory tract is able to rid itself of bacteria and what factors upset these mechanisms so that bacteria are able to take hold, colonize, and cause bronchial infection is fundamental to the entire problem of chronic bronchitis. I am also seeking aid from the National Tuberculosis Association to continue a similar study on the intratracheal administration of bacteria to animals.

/s./ Gustave A. Laurenzi, M.D.
Director of Project

/s./ Joseph F. Salerno
Business Officer of the Institution

CURRICULUM VITAE

GUSTAVE A. LAURENZI

Undergraduate School	New York University, B.A., cum laude.	1949
Medical School	Georgetown University School of Medicine, M.D., magna cum laude.	1953
Pathology	Intern in pathology, Mallory Institute of Pathology, Boston, Massachusetts	July, 1953-June, 1954
Medicine	Intern in Medicine, Yale University Medical Service, Grace-New Haven Community Hospital, New Haven, Conn.	July, 1954-June, 1955
Medicine	Assistant resident in Medicine, First (Columbia) Medical Service, Bellevue Medical Center, New York 16, New York	July, 1955-June, 1956
Pulmonary Physiology	Research fellow, Cardiopulmonary Laboratory, Columbia-Presbyterian Medical Center, New York 32, New York U.S.P.H.S. Postdoctorate fellow. Assistant Physician to Presbyterian Hospital.	July, 1956-June 1957
Pulmonary Physiology	Research fellow (same as above). Fellow in Medicine of the National Foundation for Infantile Paralysis. Assistant Physician to Presbyterian Hospital.	July, 1957-June, 1958
Clinical Chest & Medicine	Chief resident physician, Chest Service, Bellevue Medical Center, New York 16, New York. Teaching Fellow of the National Tuberculosis Association.	July, 1958-June, 1959
Bacteriology & Immunology	Research Fellow of the National Tuberculosis Association. Channing Research Fellow in Bacteriology and Immunology at Harvard, Channing Laboratory, Mallory Institute of Pathology, Boston City Hospital, Boston, Mass. Clinical Assistant in Medicine, and Chest Consultant, Second & Fourth (Harvard) Medical Services, Boston City Hospital, Boston, Mass.	July, 1959-June, 1960
	Assistant Professor of Medicine Coordinator of Division of Respiratory Diseases, Seton Hall College of Medicine, Jersey City, New Jersey	July, 1960